## REMARKS

These remarks are in response to the Office Action mailed July 13, 2001. Claims 1-18, 60, and 63 were pending before this response. By the present communication, claim 7 has been cancelled, claims 1, 5-6, 11, 15, 60 and 63 have been amended, and new claims 64-74 have been added. These amendments and additions add no new matter as the claim language is fully supported by the specification and original claims. The amendment to claim 1 is fully supported by claim 7 as filed. The amendment to claim 5 is fully supported by the disclosure as filed at page 13, lines 8-23. The other amendments are typographical in nature. Newly added claims 64-66, 68, 69, and 72 are supported by the disclosure as filed, for example, Example IV, at page 42 lines 18-19, and page 43, lines 13-16. Newly added claim 71 is supported by claim 7 as filed. Newly added claims 67 and 74 are supported by the disclosure as filed, for example at page 17, lines 28-30. Newly added claims 70 and 73 are supported by the disclosure as filed, for example Figs 2a-b and Example IV.

Reconsideration of the application in light of the foregoing amendments and the following discussion is respectfully requested.

The Office Action objected to the disclosure because of the use of the trademark "BODIPY" without acknowledgement that this is a trademark. Applicants have denoted this term as a registered trademark where it appeared in the specification, at pages 7, and 40-43.

The Office Action pointed out that the Applicant's provision of domestic priority documents 60/081, 118 filed 4/8/1998 and 60/018,340, filed 5/17/1996 appears incorrect. Applicants have corrected the application number and filing date of the priority document in amending the first paragraph of the specification above. Provisional application 60/081,340, filed April 9, 1998 was incorrectly referenced as Provisional application 60/018,340, filed 5/17/1996. Additionally, attached herewith, is a corrected declaration. A corrected, redlined filing receipt will be filed with the USPTO.

The Office Action suggested that Applicant should amend the first line of the specification to reflect the relationship of the instant application to priority document PCT US99/07847. The amendments to the first paragraph of the specification included herein, include information regarding the priority document PCT US99/07847.

The Office Action indicated that the application did not contain an abstract of the disclosure as required by 37 CFR 1.72(b). According to the Applicant's records, an abstract was included on a separate page, page 59 of the disclosure as filed. However, the Abstract has been reentered above to assure that it is part of the Patent Office file.

**The Rejection under 35 U.S.C. § 112, First Paragraph**

Applicants respectfully traverse the rejection of claims 1-18 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. Although the Office Action points out that the term "homologue" is defined on page 11 of the specification as two sequences or parts thereof that are greater than or equal to 75% identical when optimally aligned using ALIGN program, the Office Action alleges with reference to a single chain antibody that is "substantially identical" or a "homologue" to SEQ ID NO:1, that one of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species to describe the genus since it only provides one detailed sequence (SEQ ID NO:1). Thus, the Office Action concludes that Applicant was not in possession of the claimed genus, citing *University of California v. Eli Lilly and Co. 43 USPQ2d 1398* and the Revised Interim Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, 1 "Written Description" Requirement, Federal Register, Vol. 64, No. 244, pages 71427-71440, Tuesday December 2 1, 1999.

The embodiments of claims 1-18 are directed to methods for localizing a probe, which utilize a single chain antibody and the binding of this single chain antibody by a ligand. As a

preliminary matter, the Office Action's allegations regarding inadequate written description only appear to apply to claims 5 and 6 since these are the only claims which recite that the amino acid sequence of the single chain antibodies are related to SEQ ID NO:1.

Regarding claims 5 and 6, these claims have been amended to clarify that they are directed to single chain antibodies that recognize PhOx. As described in the specification as filed and known by a skilled artisan, one of ordinary skill can readily use routine techniques to develop single chain antibodies that are related to, but different in primary sequence from SEQ ID NO:1, yet retain the ability to bind PhOx. This is especially true for antibody molecules, since the precise structure of such molecules and the effects of amino acid changes on the binding properties of such molecules has been extensively studied. One or ordinary skill can readily determine the portions of the molecule that does not interact with PhOx, and can focus modifications on these portions. Furthermore, there is considerable teaching on amino acid substitutions that are conservative, and therefore, are far less likely to affect antibody binding properties.

The disclosure as filed provides sufficient written description for all the pending claims. Many single chain antibodies are known in the art. Each of these antibodies by definition binds a ligand. Therefore, based on the disclosure as filed, including the demonstration that a single chain antibody that recognizes PhOx can be successfully employed in the methods of the present invention, one of ordinary skill would recognize that virtually any single chain antibody can be used for the present invention. In other words, the characteristic of a single chain antibody necessary to be used in a method of the present invention (i.e. the ability to bind a ligand), is an inherent property of such an antibody. Therefore, all of the claimed embodiments are adequately described by the disclosure as filed.

Furthermore, the disclosure provides sufficient structural and functional information to meet the written description requirement of 35 U.S.C. 112, discussed in *Regents of the University of California v. Eli Lilly & Co.*, 119 F3d 1559, 1569 (Fed. Cir. 1997). The relevant portion of *Regents* involve claims related to vertebrate insulin cDNA and mammalian insulin cDNA, the structure of which is provided by nature. On the other hand, the methods of

pending claims 1-18, relate to single chain antibodies in general, and the ability of such antibodies to bind a ligand. The Federal Circuit in *Regents* indicated that a written description of a chemical genus requires a precise structural definition, and is not satisfied solely by functional language, such as a "vertebrate insulin cDNA." *Regents,* at 1582. Unlike the nucleic acids at issue in *Regents* whose structures are defined by nature and were unknown as of the filing date of the patents at issue, single chain antibodies used in the methods of the present invention are well-known and described in the art. In fact, virtually any of the many known single chain antibodies can by used with the present invention. The particular specificity or binding properties of the single chain antibodies are not critical to the invention. All that is required is that the single chain antibodies bind a ligand, an inherent property of a single chain antibody.

This distinction between the present application and those at issue in *Regents* applies to pending claims 5 and 6 which refer to SEQ ID NO:1, as well. Single chain antibodies with homology or substantial identity to SEQ ID NO:1 which bind PhOx, are not a genus of unknown structures that was defined by nature, as was the case for vertebrate insulin in the *Regents* case. Rather, one of ordinary skill can readily determine whether a sequence has sufficient sequence identity to meet the claimed limitation, and retains the ability to bind PhOx. Therefore, single chain antibodies that are homologues or substantially identical to SEQ ID NO:1 are adequately described in the specification. In summary, all of the pending claims are readily distinguishable from those at issue in *Regents*.

**The Rejection under 35 U.S.C. § 112, Second Paragraph**

Applicants respectfully traverse the rejection of claims 1-3, 5, 11, 13, 14, and 15 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office Action alleges that claim 1 is indefinite in the recitation of "a)" and "1), 2) and 3)" because, as written, it is unclear whether dependent claims depend on claim 1, 2, 3 or sub 1, 2, 3. The pending dependent claims all recite that they depend from a claim. Therefore, they meet the

requirements of 35 U.S.C. 112, second paragraph, in explicitly indicating that they refer to claims not subheadings. However to further clarify that the dependent claims refer to claim numbers and not subheadings, claim 1 has been amended to recite subheading i, ii, and iii in place of subheadings 1, 2, 3 in order to clarify that claims that depend from claim 1 are not dependent on subheadings. One of skill in the art would recognize this to be the intended meaning of the claims as originally presented, and accordingly the amendments do not narrow the scope of the claims.

The Office Action alleges that the phrase "as in any" renders claim 63 indefinite and ambiguous. The term "as in any" has been deleted from claim 63. Therefore the rejection is moot. One of skill in the art would recognize that the intended meaning of claim 63 has not been changed, and accordingly the amendments do not narrow the scope of the claims.

The Office Action alleges that the phrase "substantial identity" renders claim 5 indefinite. The term "substantial identity" has been deleted from claim 5. Therefore, the rejection is moot.

The Office Action alleges that the phrase "said cell" as recited in claims 11, 13, 14 and 15 has no antecedent basis in base claim 3. However, although claim 3 does not recite the term "a cell," claim 1, from which claim 3 indirectly depends, recites this term. Therefore, proper antecedent basis exists for the term "said cell" in claims 11, 13, 14, and 15.

Applicants respectfully submit, therefore, that claims 1-3, 5, 11, 13, 14, and 15 particularly point out and distinctly claim the subject matter which the applicants regard as the invention. Accordingly, reconsideration and withdrawal of the rejections of pending claims 1-3, 5, 11, 13, 14, and 15 is respectfully requested.

**The Rejection under 35 U.S.C. § 102**

Applicants respectfully traverse the rejection of claims 1-6, 8, 12-14 and 16-17 under 35 U.S.C. 102(e) as being anticipated by Chesnut (US Pat No. 6,017,754). The Office Action (citing Fig 1 A-2, Fig 6, column 1, line 54 bridging column 2 line 1, column 6, line 11) asserts that Chesnut teaches a method of identifying and selecting a cell to study genes of interest at a

cellular level by transfecting a cell with a plasmid that encode a single chain antibody (sFv) directed against phOx. The single chain antibody is related to the single chain antibody of SEQ ID NO: 1 of the present application. The Office Action further asserts that Chesnut *et al.* teach that the hapten (PhOx) as the ligand can be conjugated to a fluorescent (FITC) spectroscopic probe or other label via a linker moiety (PhOx-BSA-FITC) to allow for identification and selection of the transfected cell by detecting fluorescence emission (citing column 7, line 8-13).

Applicants respectfully assert that Chesnut does not anticipate the claimed invention. To anticipate an invention, each and every element of a claim must be found in a single prior art reference. MPEP 2131; Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628,631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Chesnut does not disclose a membrane permeant conjugate, an element recited in claim 1. Furthermore, Chesnut does not disclose a linker. Since claims 2-6, 8, 12-14, and 16-17 depend from claim 1, these claims are also not anticipated by Chesnut.

In fact, the absence of a description of a membrane permeant conjugate in Chesnut is the result of a fundamental difference between the methods of Chesnut and the present invention. Unlike the present invention which is directed to methods for localizing a probe, Chesnut focuses on methods for isolating cells that are transfected with a recombinant nucleic acid molecule. For this isolation, Chesnut describes transfecting cells with a nucleic acid capable of expressing a single chain antibody that recognizes a ligand, and isolating transfected cells using a "cell separation means" coupled to a ligand. In passing, Chesnut mentions that the ligand can also be coupled to a fluorescent moiety for detecting cells that have been isolated (Col. 7, lines 7-10). The only example of this provided by Chesnut is FITC-BSA-phOx (Col. 7, lines 7-10). Such a complex would not be expected to be membrane permeant because of the presence of the large charged BSA molecule. Furthermore, the conjugate apparently also includes a "cell separation means" (Col. 7, lines 3-10 ("this hapten or epitope is bound either directly or indirectly to a cell separation means.... The hapten or epitope *can also*

*include* or be conjugated to a fluorescent or other labeled, selectable hapten or epitope ." (emphasis added))) which would make it even less membrane permeant.

Furthermore, no linker is described for the FITC-BSA-phOx (Col. 7, lines 7-10) complex. Without any mention of a linker, one of ordinary skill would assume that the BSA molecule is directly bound to FITC and phOx. Furthermore, advantages related to increasing the distance between the probe and the ligand (See present disclosure as filed, page 17, lines 24-26) using the linker do not apply to the FITC—BSA-phOx complex because phOx, the ligand, and FITC are separated by the relatively large BSA molecule. Applicants respectfully submit, therefore, that claims 1-6, 8, 12-14 and 16-17 are not anticipated by Chesnut under 35 U.S.C. 102(b). Accordingly, reconsideration and withdrawal of the rejection of pending claims 1-6, 8, 12-14 and 16-17 under 35 U.S.C. 102(b) is respectfully requested.

**The Rejection under 35 U.S.C. § 103**

The applicants respectfully traverse the rejection of claims 1-3, 7, 11, 15, 60, and 63 under 35 U.S.C. 103(a) as being unpatentable over Chesnut, *supra*, in view of Haugland (Handbook of Fluorescent Probes and Research Chemicals 6th edition, pages 13-15, 18-19 (1996)), or Rizzuto et al. (*Current Biology* 5(6):635-42 (1995)). The Office Action concedes that the claimed invention as recited in claims 7, 11, 15, 60 and 63 differs from the references in that the probe/ligand conjugate is membrane permeant and in adding a stimulus to the cell and detecting the probe/ligand conjugate before and after addition of stimulus. However, the Office Action asserts that Haugland teaches spectroscopic probes that are membrane permeant, including BODIPY FL, and discusses at length other advantages of BODIPY FL over conventional fluorophores, as disclosed in Haugland. The Office Action asserts that Rizzuto et al. teaches a method of using recombinant green fluorescent protein (GFP) of *Aequorea victoria* as a tool for visualizing subcellular organelles in living cells using a plasmid encoding recombinant GFP, hemagglutinin HA1 epitope (membrane bound), and subunit VIII of cytochome c (mitochondria membrane), and expressing these proteins as a fusion protein in mammalian cells (HeLa cells). The Office Action further asserts that Rizzuto et al. teaches

adding a stimulus (noradrenaline and histamine) to the cell and detecting the GFP conjugate before and after addition of the stimulus. Based on these assertions, the Office Action concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute BIODIPY FL taught by Haugland or GFP taught by Rizzuto et al with the fluorecein (FITC)-BSA-phOx complexes taught by Chesnut to arrive at a method of localizing a probe in a cell expressing the probe, according to the present invention.

To establish a *prima facie* case of obviousness there must be some suggestion or motivation in the prior art to make the claimed invention, there must be a reasonable expectation of success, and the prior art reference must teach or suggest all of the claim limitations. MPEP 2142; In re Vaeck, 947 F.2d 488, 20 USPQ2d, 1438 (Fed. Cir. 1991). The mere fact that references can be combined or modified does not render the resultant combination obvious, unless the prior art also suggests the desirability of the combination. MPEP 2143.01 citing In re Mills, 916 F.2d 680 (Fed. Cir. 1990). If the proposed modification of one prior art reference would render it unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. MPEP2143.01 citing In re Gordan, 733 F.2d 900, 221 (Fed. Cir. 1984).

The cited references, Chesnut, Haugland, and Rizzuto et al., either singly or in combination, do not render obvious a method with all of the limitations of the pending claims. That is, the combination of these references does not result in a membrane permeant conjugate for detecting a single chain antibody or a specific binding pair member expressed from a recombinant nucleic acid. The Office Action indicates that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute BIODIPY FL taught by Haugland or GFP taught by Rizzuto *et al with* the fluorecein (FITC) conjugated to the phOx ligand as taught by Chesnut *et al*" for a method of localizing a probe in a cell expressing the probe. However, such a combination would not result in a membrane permeant conjugate, as recited in the pending claims. As mentioned above, it is the BSA moiety and the cell-separation means of the FITC-BSA-PhOx complexes discussed in Chesnut that render them membrane impermeable, not FITC. In fact, Example IV, and FIG. 2B illustrate that FITC can

be successfully employed in the conjugates of the present invention. Therefore, inclusion of the probes disclosed in Haugland or Rizutto et al. would not render the conjugates membrane permeant. Furthermore, as discussed above, the conjugates of Chesnut appear to further include a cell-separation means, which also render them membrane impermeant. This cell separation means is central to the methods of Chesnut, even when considering Haugland and/or Rizutto et al.

Furthermore, a conjugate containing green fluorescent protein (GFP) would not be membrane permeant. GFP is used by Rizzuto for intracellular localization because it is expressed within the cell, not because it is membrane permeant. Large charged molecules such as proteins are not membrane permeant. Therefore, substituting GFP for FITC in the conjugates of Chesnut would result in a conjugate with 2 large charged proteins, BSA and GFP, which would render the conjugate even more membrane impermeant.

Furthermore, Haugland and Rizzuto et al. do not suggest or motivate one of ordinary skill to make the present invention because even if the method of Chesnut is modified with the teachings of Haugland and/or Rizzuto et al. to provide a membrane permeant conjugate, as indicated in the Office Action, and the Applicants reassert their position that they would not, a membrane permeant conjugate would render the method of Chesnut unfit for its intended purpose. The methods of Chesnut involve isolation of cells by binding a complex containing a hapten or epitope and a cell separation means to a single chain antibody that is expressed on the cell's surface (Col. 6, lines 13-15). The use of a membrane permeant conjugate with the methods of Chesnut would render such methods unfit for their intended purpose, because such conjugates could not be used for cell isolation according to the methods described in Chesnut In fact, Chesnut teaches away from using a membrane permeant conjugate to detect an expressed single chain antibody in indicating that the single chain antibody is expressed on the surface to act as a "molecular hook" to allow isolation of a transfected cell (Col. 6, lines 13-15). There is no advantage, and in fact, there would probably be undesirable effects, of using a conjugate that is membrane permeant with the methods of Chesnut. It is improper to combine

references where the references teach away from their combination. In re Grasselli, 713 F.2d 731, 743, 218 USPQ 769, 779 (Fed. Cir. 1983).

In addition to the deficiencies discussed above, the cited prior art references do not teach a conjugate wherein a ligand is bound to a probe via a linker. The linker of the present invention functions as a spacer between the probe and the linker to prevent sterric interference of the probe by the ligand, upon binding of the single chain antibody (See application as filed page 17, lines 24-26). However, Chesnut discloses complexes wherein BSA is localized between an epitope or hapten and a single chain antibody. Therefore, a large distance between the epitope or happen and the probe is present, thereby eliminating the advantage of a non-ionic linker, as discussed in the present invention.

It is noteworthy that newly added claims 64-66, 68-69, and 72-73, directed to methods whereby the probe provides a more intense signal when the probe/ligand conjugate is bound than unbound, capture a further distinction between the present invention and the methods of Chesnut which are not overcome by the teachings of Rizzuto et al. and Haughland. As disclosed in the present specification (e.g. Example 4, pg. 42 lines 18-19, page 43, lines 13-16), the present invention provides several examples of conjugates that have significantly more fluorescence when they are bound than when they are unbound. Chesnut on the other hand focuses on ligands that are bound to a large cell separation means and also, as mentioned in passing, can be bound to a second ligand that is labeled. Because of the increased separation between the label which is attached to the second ligand and the first ligand, as well as the fact that the probe is attached to the second ligand, not the first, one of ordinary skill would not expect that fluorescence would be increased on binding of the first ligand to its binding pair member. Neither Rizzuto et al. nor Haugland overcome this deficiency, since the teachings of these prior art references do not suggest to one of ordinary skill to eliminate a ligand in the methods of Chesnut and to directly connect a probe to a ligand using a linker that provides increased fluorescence upon binding of the ligand to a specific binding partner.

It is noteworthy regarding newly added claims 67 and 74 which recite that the linker is a flexible aliphatic linker or a rigid aromatic linker, that there is no motivation or suggestion to

use such linkers with the method of Chesnut, even when considering the teachings of Haugland and Rizzuto et al. The linkers provide improved performance of the methods of the present invention by yielding conjugates with improved fluorescence and/or increased fluorescence upon binding to a specific binding pair member such as a single chain antibody (See e.g., page 17, lines 24-26; page 42, lines 8-10; and page 42, lines 18-19). As discussed above, Chesnut on the other hand discusses complexes in which a ligand is bound to a large cell separation means and also, as mentioned in passing, optionally to a second ligand that is labeled. Because of the increased separation between the label, when present, which is attached to the second ligand and the first ligand, one of ordinary skill in the art would not expect that fluorescence properties would be improved by the use of the linkers of the present invention. Neither Rizzuto et al. nor Haugland can overcome this deficiency of the primary reference because neither reference suggests that the fluorescent properties of a conjugate can be improved by binding the probe directly to the first ligand using a a flexible aliphatic linker or a rigid aromatic linker

It is noteworthy regarding newly added claims 70-74 that the conjugate of the claims "consists essentially of" or "consists of" (claim 73) the probe, ligand, and non-ionic linker. Chesnut involves ligands that are bound to a "cell separation means such as magnetic beads or sheets, tubes, porous matrices, or any natural or synthetic material including metals, polymers, latex beads, agarose, Sepharose, or any solid surface" (Col. 7, lines 3-7). Chesnut mentions, as discussed above, that the ligand "can also include or be conjugated to a fluorescent or other labeled, selectable hapten or epitope. The only example of such a connjugate is PhOx-BSA-FITC." (Col. 7, lines 7-10). Such a conjugate that includes a ligand, a second hapten or epitope (i.e. a second ligand), and a label does not meet the limitations of these claims since they include a second ligand (e.g. BSA). Furthermore, the PhOx-BSA-FITC conjugate apparently is bound to a cell separation means, further distinguishing it from the claimed invention of claims 70-74. Modifying such a conjugate to remove the cell separation means and the second hapten or epitope, would render the method of Chesnut unfit for its intended purpose since such a conjugate would not allow isolation of cells by a physical means according to the

teachings of Chesnut. Neither Rizzuto et al. nor Haugland can overcome this deficiency since the primary reference relied on in the Office Action is inoperable by the modification to eliminate the second hapten and/or the cell separation means from the conjugate.

The Office Action rejected claim 9 under 35 U.S.C. 103(a) as being unpatentable over Chesnut (US Pat No. 6,017,754) in view of Haugland (Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-15, 18-19) as applied to claims 1-3, 7, 11, 15, 60 and 63 and further in view of Lauffer et al (U.S. Pat No. 5,628,982). The Office Action asserts that the claimed invention of claim 9 differs from Chesnut and Haugland only by the recitation that detecting is by means of NMR imaging. The Office Action asserts that Lauffer et al. teaches hydroxyl-aryl metal chelates as NMR contrast agents or probes for diagnostic NMR imaging, and alleges that it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute hydroxy-aryl metal chelates as NMR contrast agents taught by the Lauffer et al., with the BIODIPY FL probe taught by Haugland or the fluorecein (FITC) probe conjugated to the phOx ligand as taught by Chesnut for NMR imaging. Furthermore, the Office Action alleges that one would have been motivated, with a reasonable expectation of success, to substitute hydroxy-aryl metal chelates as NMR contrast agents taught by Lauffer et al., for NMR imaging because the gadolinium ion with seven unpaired electrons can be used with any chelating agent having a number of open sites; it can act as a contrast agent at very low dosages and is no more toxic than iron when used with a chelating agent having no open sites as taught by Lauffer et al.

Although Lauffer et al. discusses NMR contrast agents, it does not overcome the deficiencies of Chesnut and Haugland either individually or in combination. As described above, the combination of Chesnut and Haugland does not result in a method which utilizes a membrane permeant conjugate for detecting a single chain antibody or a specific binding pair member expressed from a recombinant nucleic acid. Furthermore, as described above, Chesnut, the primary reference, is rendered unsuitable for its intended purpose if the conjugate

used therein is membrane permeant. As is the case for Haugland, nothing in Lauffer et al. changes these conclusions.

The use of the NMR enhancing agents of Haugland, with the two-ligand labeled conjugates of Chesnut would not change the fact that such conjugates are not membrane permeant. As discussed above, this impermeable property is the result of the presence and size of the second ligand and the cell separation means and not the probe. Therefore, changing the nature of the probe, even by using a more membrane permeable probe, would not make the conjugate membrane permeable.

Furthermore, there is no suggestion or motivation to combine Lauffer et al. with Chesnut As discussed above, Chesnut is directed to methods of isolating cells using cell separating means. Lauffer et al. does not involve and is not related to a cell separation means. Therefore, one of ordinary skill in the art would not be motivated to combine Lauffer et al. with Chesnut Furthermore, Lauffer et al. does not indicate, and one of ordinary skill would not expect success without actually performing experiments attempting to obtain single chain antibodies that bind the small organic ligands disclosed in Lauffer et al., as required for claim 9. Finally, the NMR contrast agents disclosed in Lauffer et al., are complexes of certain metal ions and certain ligands. As such, the complexes do not include a linker, as recited in the pending claims.

The Office Action rejected claim 10 under 35 U.S.C. 103(a) as being unpatentable over Chesnut (US Pat No. 6,017,754, filed Aug 1995, PTO 892) in view of Haugland (Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-1 5, 18-19) as applied to claims l-3, 7, 11, 15,60 and 63 and further in view of Green et al (U. S. Pat No. 5,324,502). The Office Action asserts that the claimed invention of claim 10 differs from Chesnut and Hagland et al. only by the recitation of the detecting by positron emission tomography. The Office Action asserts that Green et al. teaches radiopharmaceuticals for positron emission tomography (PET) wherein the radiopharmaceutical is a positron emitting gallium-68(III) cationic complex or lipophilic complex which is membrane permeant due to its lipophilic

content. The Office Action alleges that it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the positron emitting gallium-68(1II) cationic complex or lipophilic complex taught by the Green et al. patent with the BIODIPY FL probe taught by Haugland or the fluorecein (FITC) probe conjugated to the phOx ligand as taught by Chesnut for PET as taught by Green et al.

Although Green et al. discusses radiopharmaceuticals for PET, it does not overcome the deficiencies of Chesnut patent and Haugland reference either individually or in combination. As described above, the combination of the Chesnut patent and Haugland reference does not result in a method which utilizes a membrane permeant conjugate for detecting a single chain antibody or a specific binding pair member expressed from a recombinant nucleic acid. Furthermore, as described above, Chesnut, the primary reference, is rendered unsuitable for its intended purpose if the conjugate used therein is membrane permeant. Nothing in Green et al. changes these conclusions. The use of the radiopharmaceuticals for PET of Green et al., with the two-ligand labeled conjugates of Chesnut which also appear to include a cell-separation means, would not change the fact that such conjugates are not membrane permeant. As discussed above, this impermeable property is the result of the presence and size of the second ligand and the cell separation means and not the probe. Therefore, changing the nature of the probe, even by using a more membrane permeable probe, will not make the conjugate membrane permeable.

Furthermore, there is no suggestion or motivation to combine Green et al. with Chesnut. As discussed above, Chesnut is directed to methods of isolating cells using cell separating means. Green et al. does not involve and is not related to a cell separation means. Therefore, one of ordinary skill in the art would not be motivated to combine Green et al. with Chesnut Furthermore, Green et al. does not indicate, and one of ordinary skill would not expect success without actually performing experiments attempting to obtain single chain antibodies that bind the metal chelating ligands disclosed in Green et al. Finally, the radiopharmaceuticals for PET disclosed in Green, are complexes of metal chelating ligands and

certain radioactive metals. As such, the complexes do not include a linker, as recited in the pending claims.

The Office Action rejected claim 18 under 35 U.S.C. 103(a) as being unpatentable over Chesnut (US Pat No. 6,017,754) in view of Haugland (Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-15, 18-19) as applied to claims 1-3, 7, 11, 15,60 and 63 and further in view of Youn et al (Analytical Biochemistry 232: 24-30, 1995). The Office Action asserts that the claimed inventions of these claims differs from the teachings of the Chesnut patent and the Haugland reference by measuring fluorescence anisotropy of the probe. The Office Action indicates that Youn et al. teaches the use of fluorescence energy transfer immunoassay (FRET) based on the use of a ruthenium metal ligand complex, and that it would have been obvious for one of ordinary skill in the art at the time the invention was made to substitute the ruthenium metal ligand complex probe taught by Youn et al. with the BIODIPY FL probe taught by Haugland or the fluorecein (FITC) probe conjugated to the phOx ligand as taught by Chesnut for a FRET assay. The Office Action alleges that one would have been motivated, with a reasonable expectation of success, to substitute the ruthenium metal ligand complex taught by Youn et al because the Ru complex is its long decay time, chemically and photochemically stable which allows off-gating of the interfering autofluorescence and thereby increases the sensitivity of the time-resolved immunoassays.

Although Youn et al. discusses FRET immunoassays, it does not overcome the deficiencies of Chesnut and the Haugland reference, either when taken individually or in combination. As described above, the combination of the Chesnut patent and the Haugland reference does not result in a method which utilizes a membrane permeant conjugate for detecting a single chain antibody or a specific binding pair member expressed from a recombinant nucleic acid. Furthermore, as described above, Chesnut, the primary reference, is rendered unsuitable for its intended purpose if the conjugate used therein is membrane permeant. As is the case for Haugland, nothing in Youn et al. changes these conclusions. The use of the FRET immunoassays of Youn et al., with the two-ligand labeled conjugates of

Chesnut which also appear to include a cell-separation means, would not change the fact that such conjugates are not membrane permeant. As discussed above, this impermeable property is the result of the presence and size of the second ligand and/or the cell separation means, and not the probe. Therefore, changing the nature of the probe, even by using a more membrane permeable probe, will not make the conjugate membrane permeant.

Furthermore, there is no suggestion or motivation to combine Youn et al. with Chesnut. As discussed above, Chesnut is directed to methods of isolating cells using cell separating means. Youn et al. does not involve, and is not related to a cell separation means. Therefore, one of ordinary skill in the art would not be motivated to combine Youn et al. with Chesnut Furthermore, the immunoassays of Youn et al. cannot be successfully combined with the methods of Chesnut because the immunoassays of Youn et al. require a close interaction of donor and acceptor antibody/antigen pairs, and provide a change in signal in response to binding of receptor labeled antibody and donor labeled antigen. However, as discussed above, the methods of Chesnut which utilize a fluorescent label, label a second ligand and not the ligand that interacts with the expressed single chain antibody. Therefore, binding of the single chain antibody to its ligand on the 2-ligand conjugates, cannot be easily modified to provide the acceptor/donor interaction, FRET assays of Youn et al.. Furthermore, the immunoassays of Youn et al. require a labeling of both donor and acceptor, which is not compatible with the methods of Chesnut. The methods of Chesnut require that a single chain antibody is expressed on the surface of a living cells. This does not appear to be compatible with the methods of Youn et al. in which an isolated antibody is labeled with the RB4 acceptor *in vitro*. Finally, the FRET immunoassays of Youn et al. utilize complexes of donor and ligand that do not include a linker, as recited in the pending claims. Applicants respectfully submit, therefore, that claims 1-3, 7, 9, 11, 15, 18, 60 and 63 are not obvious in view of Chesnut in view of Haugland, Rizzuto et al., Lauffer et al., Green et al, and Youn et al.

In view of the above remarks, reconsideration and favorable action on all claims is respectfully requested. Should any questions remain in view of this communication, the

Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved. Please charge any additional fees, or make any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

Date: December 13, 2001

Lisa A. Haile, J.D., Ph.D.
Reg. No. 38,347
Attorney for Applicant
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

Customer Number 28213
Gray Cary Ware & Freidenrich LLP
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133

**EXHIBIT A**

**VERSION WITH MARKINGS**

**IN THE SPECIFICATION**

Please amend the specification as follows:

**At page 7, please replace the paragraph at lines 4-19 with the following paragraph:**

**FIG. 3** represents site-specific labeling of CHO cells. **FIG. 3A** is a confocal image of CHO cells transfected with the plasma membrane-targeted sFv vector and shows plasma membrane staining by phOx-rhodamine. **FIG. 3B** is a confocal fluorescence image of the cells in **FIG. 3A** stained with a fluorescein-labeled anti-c-myc antibody. **FIG. 3C** shows the brightfield image of the cells in **FIG. 3A**. **FIG. 3D** shows a fluorescence image of CHO cells with Golgi-targeted sFv in the presence of 10 nM phOx-Bodipy®F1. **FIG. 3E** shows the same cells as in **FIG. 3D** in the presence of 500 nM phOx-ethanolamine and 10 nM phOx-Bodipy®FL. **FIG. 3F** is a fluorescence image of a cell with ER-targeted sFv in the presence of 10 nM phOx-Bodipy®. **FIG. 3G** shows immunostaining of Golgi-sFv transfected cells with fluorescein labeled, anti-c-myc antibody. **FIG. 3H** shows immunostaining of Golgi-sFv transfected cells with rhodamine-labeled anti-mouse antibody directed against a mouse 58k protein antibody. **FIG. 3I** shows immunostaining of ER-sFv transfected cells with rhodamine-labeled anti-mouse antibody directed against an anti-c-myc antibody. **FIG. 3J** shows immunostaining of ER-sFv transfected cells with fluorescein-labeled concavalin A. The scale bar represents 10 micrometers.

**At pages 40-41, please replace the paragraph at line 14 of page 40 to line 3 of page 41 with the following paragraph:**

A flexible aliphatic linker was added to 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (phOx) (Sigma) by reaction of 50 mg phOx with 14.3 µl 1,5-diaminopentane (Aldrich) in 2.5 ml acetone for 1 h. The di-substituted aminopentane was precipitated by addition of two volumes of 50 mM borate buffer (pH 9.2) leaving the product in solution. A more rigid trans-

cyclohexane linker was added to phOx by reaction of trans-1,4-diaminocyclohexane (Aldrich) (5.1 mg in 0.5 ml of DMSO) and phOx (10 mg in 0.5 ml acetone) for 2 h. The di-substituted diaminocyclohexane was precipitated by addition of an equal volume of water, leaving the monosubstituted diaminocyclohexane in solution. PhOx-Bodipy® Fl was prepared by reaction of excess Bodipy® FL succinimidyl ester (Molecular Probes) with 1 mM of phOx-aminopentane in borate buffer for 2 h. The product was obtained as a precipitate. PhOx-tetramethylrhodamine was prepared by reaction of excess tetrametholrhodamine succinimidyl ester (Molecular Probes) with 5 mM phOx-aminocyclohexane in borate buffer for 6 h. The product was obtained as a precipitate. PhOx-fluorescein was prepared by reaction of equimolar amounts of phOx (0.5 mg in 25 µl acetone) and fluorescein cadaverine (Molecular Probes) (1 mg in 50 µl dimethylformamide) for 1 h. PBS was added, unreacted phOx was removed by hexane extraction, and the product was extracted with butanol. PhOx-ethanolamine was prepared by reaction of 1 mg phOx with 0.3 µl ethanolamine in 10 ml ethanol for 1 h. All reactions were conducted at room temperature. Products were judged to be greater than 95% pure by TLC and compounds were confirmed by mass spectrometry using well-known methods.

**At pages 41-42, please replace the paragraph at line 24 of page 41 to line 10 of page 42, with the following paragraph:**

A cell labeling method was developed that combines the site specificity conferred by genetically encoded protein targeting sequences with the spectral and indicator properties of fluorophores. The strategy is to express a high affinity specific binding partner at a specified intracellular location to trap a conjugate of an indicator linked to a specific ligand (**FIG. 2A**). We chose a single-chain antibody (Chesnut, supra, 1996) (sFv) as the specific binding partner, and a high affinity hapten (phOx) as the ligand. Although many receptor-ligand pairs are possible, the antibody-hapten pair used here was chosen because of the simple ligand-probe chemistry and high affinity interaction without interference from cellular factors. For sFv targeting, cells are transfected with cDNAs encoding sFv in fusion with Golgi, ER or plasma

membrane-targeting sequences. Fluorophore-hapten conjugates were added to the extracellular solution at low concentrations, diffused to sites of sFv expression, and bound to the sFv. Conjugates of different indicator, spectral and linker properties were synthesized (**FIG. 2B**), including phOx-Bodipy® FL (green fluorescent, flexible linker), phOx-fluorescein (green fluorescent, pH-sensitive, flexible linker), and phOx-tetrametholrhodamine (red fluorescent, rigid linker). The flexible linkers were designed to permit stacking of the unbound hapten with its covalently attached fluorophore to form a dark (non-fluorescent) complex.

**At page 43, please replace the paragraph at lines 4-19 with the following paragraph:**

The various sFv targeting constructs and phOx conjugates were studied in CHO cells. **FIG. 3A** shows a fluorescence image of living cells expressing the sFv at the plasma membrane and stained with phOx-rhodamine. A plasma membrane staining pattern was found. **FIG. 3B** shows staining of sFv in the same cells with a fluorescein-labeled anti-c-myc antibody. Comparison with **FIG. 3A** demonstrates that only sites of sFv expression were significantly labeled with phOx-rhodamine. There was no significant staining of adjacent cells that did not express sFv (**FIG. 3C**). **FIG. 3D** shows specific phOx-Bodipy® staining of Golgi. Staining was reversed by addition of 1 μM phOx-ethanolamine (**FIG. 3E**). **FIG. 3F** shows phOx-Bodipy® staining of ER, seen as a characteristic reticular pattern. The high expression level of the sFv, the relatively high affinity of the hapten/sFv, and the low fluorescence of the unbound conjugate allowed images to be obtained in the presence of < 10 nM concentrations of unbound conjugate with little contribution from free conjugate. For quantitative measurement of organelle pH, the free dye was washed out of the bathing solutions. Leakage out of the Golgi, which required dissociation from the sFv and diffusion through lipid membranes and unstirred layers, had a half time of tens of minutes.

**On page 43, please replace the paragraph at lines 20-31 with the following paragraph:**

The subcellular location of expressed sFv was confirmed by immunofluorescence. Cells transfected with the Golgi-sFv construct showed perinuclear staining by a fluorescein-labeled, anti-c-myc antibody (**FIG. 3G**) which co-localized with staining by antibodies against the Golgi marker 58k protein (**FIG. 3H**). Cells transfected with the ER-sFv construct showed a reticular staining pattern with the c-myc antibody (**FIG. 3I**) which co-localized with staining by fluorescein labeled concavalin A, an ER marker (**FIG. 3J**). The membrane permeability of the conjugates was high enough to load cells by incubation at 37°C for 4 hours for phOx-fluorescein, 2 hours for phOx-rhodamine, or 10 minutes for the less polar phOx-Bodipy®. Cells could be loaded at 4°C, indicating that the conjugate entered the cells primarily by transmembrane diffusion and not by endocytosis. These results demonstrate the selective targeting of fluorescent probes to expressed sFv in living cells.

**IN THE CLAIMS**

Please cancel claim 7.

Please amend the claims as follows:

1. (Amended) A method for localizing a probe, comprising:

a) contacting a sample comprising a cell expressing a single chain antibody with a membrane permeant probe/ligand conjugate, said probe/ligand conjugate comprising:

i) [1)] a probe moiety,

ii) [2)]a ligand that can bind with said single chain antibody, and

iii) [3)]a linker moiety coupling said probe to said ligand.

5. (Amended) The method of claim 2, wherein said single chain antibody has at least 30% sequence identity [substantial identity] to SEQ.ID.No. 1 and is capable of recognizing PhOx.

6. (Amended) The method of claim 2, wherein said single chain antibody is a [an] homologue of SEQ.ID.No. 1 and is capable of recognizing PhOx.

11. (Amended) The method of claim 3, wherein said detecting comprises locating said fluorescence characteristic of said fluorescent moiety within [with] said cell.

15. (Amended) The method of claim 3, further comprising the steps of,

i) [1)] adding a stimulus to said cell and

ii) [2)] detecting said probe/ligand conjugate, before and at least one time after addition of said stimulus.

60. (Amended) A method for localizing a probe, comprising:

b) contacting a sample comprising a cell expressing a specific binding partner with a probe/ligand conjugate, said probe/ligand conjugate comprising:

i) [1] a probe moiety,

ii) [2] a ligand that can bind with said specific binding partner, and

iii) [3] a linker moiety coupling said probe to said ligand,

wherein said ligand and said specific binding partner bind non-covalently, wherein said probe/ligand conjugate is membrane permeant, and wherein the specific binding partner is expressed from a recombinant nucleic acid.

63. (Amended) The method [as in any of] according to claims 60, 61 or 62, wherein the specific binding partner is a single chain antibody.

Please add the following claims:

--64. The method of claim 1, wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when it is unbound.

65. The method of claim 2, wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when is unbound.

66. The method of claim 1, wherein the probe is a fluorescent probe that is at least about 5-fold less fluorescent in an unbound versus bound state.

67. The method of claim 1, wherein the linker is a flexible aliphatic linker or a rigid aromatic linker.

68. The method of claim 60, wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when it is unbound.

69. The method of claim 60, wherein the probe is a fluorescent probe that is at least about 5-fold less fluorescent in an unbound versus bound state.

70. A method for localizing a probe, comprising:

a) contacting a sample comprising a cell expressing a single chain antibody with a probe/ligand conjugate, said probe/ligand conjugate consisting essentially of:

i) a probe moiety,

ii) a ligand that can bind with said single chain antibody, and

iii) a non-ionic linker moiety coupling said probe to said ligand.

71. The method of claim 70, wherein said probe/ligand conjugate is membrane permeant.

72. The method of claim 70, wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when it is unbound.

73. The method of claim 70, wherein the probe/linker conjugate consists of elements i), ii), and iii).

74. The method of claim 70, wherein the linker is a flexible aliphatic linker or a rigid aromatic linker.--